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MUSCLE FILAMENT SPACING AND SHORT-TERM HEAVY-RESISTANCE EXERCISE IN HUMANS

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SUMMARY

1. Six weeks of a dynamic heavy-resistance training of the quadriceps muscle in healthy young men resulted in a continuous increase in muscle strength, in an increase in muscle cross-sectional area (significant only in the second half of the training period) and in an increase in radiological density of the muscle tissue of 3·1% ($2P < 0·001$) in the first three weeks and 1·6% ($2P < 0·01$) in the second three weeks.

2. The linear distance between myosin filaments (38·7 ± 0·3 nm before, 38·7 ± 0·4 nm after training; mean ± S.E.M.) as well as the ratio of actin to myosin filaments (3·94 ± 0·03 before, 3·86 ± 0·06 after training) did not change with training.

3. These results refute the concept that the increases in muscle strength or radiological density during short-term heavy-resistance training are caused by changes in myofilament spacing.

INTRODUCTION

It has been shown that during the first few weeks of heavy resistance training, muscle strength increases to a greater extent than muscle cross-sectional area (Moritani & DeVries, 1979; Lüthi, Howald, Claassen, Rösler, Vock & Hoppeler, 1986). This gain in muscle strength is associated with a small increase in radiological density of muscle tissue (Horber, Scheidegger, Grünig & Frey, 1985; Jones & Rutherford, 1987). Jones & Rutherford (1987) offered a number of possible explanations of the changes in radiological density with strength training, including changes in fat content, connective tissue and packing density of the myofilaments. In support of the latter possibility, Penman had reported in 1970 a decrease in the distance between myosin filaments following heavy-resistance training in humans, interpreted as an increase in myofilament packing density.

In a previous study on controlled training in humans we had combined the study of cellular changes by muscle biopsy with a radiological examination of muscle mass changes by computer tomography (Lüthi *et al.* 1986). The material from this study

Authors' names are in alphabetical order.

was now used to explore whether the packing density of the myofilaments changes during short-term heavy-resistance training and whether such a change could quantitatively be related to the changes in strength and radiological density of the muscle tissue.

METHODS

Informed consent was obtained from eight healthy men (average age = 18 years, range 16–20; weight = 68 kg, range 64–78; height = 1·84 m, range 1·76–1·94). The experimental protocol was approved by the local ethical committee of the medical faculty. The training schedule and assessment of strength gain have been published previously (Lüthi *et al.* 1986).

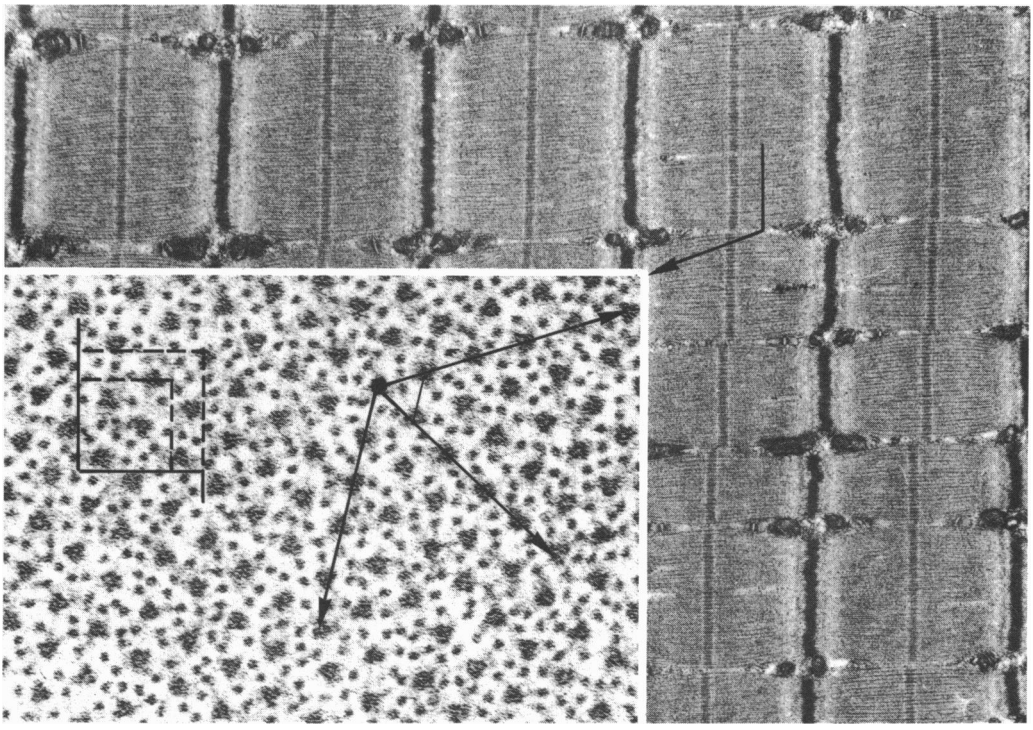

Fig. 1. Electron microscopy images of portions of human vastus lateralis muscle fibres: longitudinal section at a magnification of ×13000; cross-section at A-band level at a magnification of 170000. Squares of the test grid for counting the myofilaments (large square for myosin; small square for actin filaments) are superimposed on the cross-section part of the muscle fibre. Myosin filaments (large dots) are arranged in equilateral triangles. Arrows indicate directions for measuring linear distance between myosin filaments.

The cross-sectional area of the thigh was measured planimetrically (MOP AMO3, Kontron AG, Zürich, Switzerland) on computerized tomography scans (125 kVp, 5 s, 230 mAs; Somatom SF, Siemens, Erlangen, Germany) obtained at mid-thigh level (Lüthi *et al.* 1986). Radiological density of the quadriceps femoris muscle was calculated as described by Bulcke, Termote, Palmers & Crolla (1979) and Jones & Rutherford (1987) by averaging the mean Hounsfield number from three discrete sites in the quadriceps muscle (m. intermedius, m. rectus, m. vastus lateralis).

Muscle biopsies of the vastus lateralis were taken at the mid-thigh level before and after training using the technique of Bergström (1962). A fraction of the muscle tissue sample from each subject was processed for electron microscopy by fixation in a 6·25% solution of glutaraldehyde (Hoppeler,

Mathieu, Krauer, Claassen, Armstrong & Weibel, 1981). The actin to myosin ratio and the distance between myosin filaments were calculated from a sample of ten transversely cut muscle fibres per biopsy, at a final magnification of × 190000 (twenty micrographs; Fig. 1). A test grid with twenty squares of 0·011 μm^2 for myosin and of 0·006 μm^2 for actin was superimposed onto each micrograph. In two randomly chosen squares, in which myofilaments were cut in perfect cross-section, actin and myosin filaments were counted according to the rules of Gundersen (1977; about 800 actin and 360 myosin per biopsy; Fig. 1). From these results, actin to myosin ratio and myosin packing density (number of myosin profiles per unit area) were calculated. The mean linear distance between myosin filaments was calculated from the myosin packing density under the assumption that the myosin filaments are arranged in an equilateral triangle (Fig. 1). The average distance between myosin filaments was additionally assessed by measuring the distance across five myosin interspaces in all three directions of the triangular lattice starting at a randomly chosen point (Fig. 1; 300 distances between myosin filaments for each biopsy). The sarcomere length was analysed at a final magnification of 3600 in a subsample of ten micrographs of longitudinally cut muscle fibres of the same four tissue blocks by turning them 90 deg (Fig. 1). On each micrograph, ten consecutive sarcomere lengths were measured (100 sarcomere lengths for each biopsy) and averaged.

For statistical comparison of group means (before and after training), Student's *t* test for paired samples was used. The level of statistical significance was set at 5%. Linear least-squares regression analysis (with the assumption of a normal distribution of errors) was used to describe relationships between parameters. If variables did not change significantly with strength training, pre-training values are reported.

RESULTS

The results of strength gain, muscle cross-sectional area, radiological density and myofilament spacing are summarized in Table 1. Heavy-resistance training resulted in an increase of 18% in quadriceps isokinetic torque ($P < 0·01$) and of 11% in muscle cross-sectional area of the thigh ($P < 0·001$).

Ultrastructure of muscle tissue

There was no significant difference in sarcomere length before and after training (2·02 ± 0·02 μm, mean ± S.E.M.; Table 1). Therefore, a correction of the measured distance between myosin filaments, known to depend on sarcomere length (Brandt, Lopez, Reuben & Grundfest, 1967), was not necessary. The mean linear distance between myosin filaments was similar before and after training when estimated from measurements of packing density (38·4 ± 0·34 nm; mean ± S.E.M.; Table 1) or from direct measurements of the intermyosin filament distance (38·7 ± 0·30 nm, mean ± S.E.M.). We also found no significant changes in the actin to myosin ratio with training (3·94 ± 0·03, mean ± S.E.M.; Table 1). As reported in a previous paper, volume densities of myofibrils, lipids and sarcoplasm remained constant, whereas the volume density of mitochondria decreased significantly 9·6% ($P < 0·05$; Lüthi *et al.* 1986).

Radiological density of muscle tissue

There was a small increase in radiological density with training (Table 1). During the first three weeks of training, the mean Hounsfield number increased 3·1% and during the second three weeks another 1·6%. These increases were statistically significant ($2P < 0·01$–$0·001$) because of very small interindividual sampling variance.

TABLE 1. Results of strength gain, muscle cross-sectional area, radiological density and ultrastructural analysis before and after six weeks of heavy-resistance training

		Quadriceps torque (N m)	Muscle cross-sectional area (cm^2)	Hounsfield units (HU)
Before	Mean	125·1	149·7	60·48
	S.E.M.	5·86	4·92	0·25
After	Mean	148·0*	165·7†	63·37‡
	S.E.M.	7·84	4·73	0·12

		Actin : myosin	Sarcomere length (μm)	Myosin distance (nm)	Myosin density ($n/\mu m^2$)
Before	Mean	3·94	2·02	38·7	1604
	S.E.M.	0·03	0·02	0·3	145
After	Mean	3·86	1·97	38·7	1599
	S.E.M.	0·06	0·03	0·4	142

Means: * $P < 0·01$, † $P < 0·001$, ‡ $2P < 0·001$, paired Student's t test. Quadriceps torque: isokinetic torque at 30 deg (0 deg full knee extension) at an angle velocity of 60 deg/s. Muscle cross-sectional area: measurements at mid-thigh level. Hounsfield units: radiological density for quadriceps muscle. Myosin density: number of myosin filament profiles per μm^2 myofibril cross-sectional area.

DISCUSSION

Our observation of a small, but statistically significant increase in radiological density of muscle tissue in response to strength training is consistent with reports of other authors (Horber *et al.* 1985; Jones & Rutherford, 1987). Jones & Rutherford (1987) discussed three possible reasons for the observed increase in radiological density: (1) an increase in connective tissue, which has been reported by various authors (Schiaffino, Bormioli & Aloisi, 1972; Goldberg, Etlinger, Goldspink & Jablecki, 1975; MacDougall, Sale, Alway & Sutton, 1984); (2) a decrease in fat content of the muscle tissue, which has been correlated to radiological density of muscle tissue by other investigators (Jones, Round, Edwards, Grindwood & Tofts, 1983; Newham, Harrison, Tomkins & Clark, 1988); an increase in packing density of the contractile elements. This latter possibility appears supported by the report of Penman (1970) that the intermyosin filament distance decreased with strength training ($47·7 \pm 0·1$ nm before, $41·5 \pm 0·1$ nm after training; mean ± S.E.M.); this author further found a decrease in the number of actin filaments around a myosin filament ($8·95 \pm 0·16$ before, $7·65 \pm 0·10$ after training), which he interpreted as a change in the ratio of actin to myosin filaments.

The results of the present study, which concerned the third explanation mentioned by Jones & Rutherford (1987), refute Penman's data (1970): there was no change in the ratio of actin to myosin; the distance between myosin filaments remained constant; and therefore the packing density of the myofilaments was not changed. The disparate results of the two studies may be due to different methodological, especially morphological, approaches or to differences in the training regime. Penman's method for calculating actin to myosin ratio depended on simply counting the actin filaments around a myosin filament. This method is imprecise, because individual actin filaments cannot properly be assigned to any specific filament as evident in Fig. 1. Penman did not describe precisely enough how and by what

sampling strategy the distance between myosin filaments was measured. In order to calculate the myosin filament density, the distance between myosin filaments and the actin to myosin ratio, we used an unbiased sampling method and counted the number of actin and myosin profiles per unit area of myofibrillar cross-section, using established stereological rules (Gundersen, 1977).

In conclusion, we found that heavy-resistance exercise does not change either the packing density of the myofilaments or the ratio of actin to myosin filaments. These results refute the concept that increases in strength and in radiological density of muscle tissue with heavy-resistance training are caused by changes in muscle filament spacing.

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